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Respiratory Changes Associated With Victoria Blight of Oats.

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RESPIRATORY CHANGES ASSOCIATED WITH VICTORIA BLIGHT OF OATS

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ABSTRACT

Helminthosporium victoriae, the causal organism of Victoria blight of oats, produces prominent streaking and striping of the leaves of susceptible plants, but the fungus is usually restricted to the base of the culm and usually cannot be isolated from areas of prominent symptoms. Previous investigators proposed that a toxin produced by the fungus was responsible for the production of symptoms. The presence of such a toxin that is as specific as the organism that produced it, was demonstrated by several workers who extracted it from culture filtrates of the fungus. This toxin was given the trivial name, victorin. In several studies it has been reported that prominent respiratory changes take place in susceptible plants when they are treated with victorin.

In this study an attempt was made to determine whether respiratory changes, comparable to those induced by victorin, could be found in inoculated plants as well. A gradual increase in respiratory rate and a corresponding decrease in response to 2,4-dinitrophenol were observed in inoculated plants. In homogenates prepared from inoculated plants an increase in ascorbate oxidation occurred, with maximum activity occurring after the respiratory maximum of whole tissue was reached.

Dinitrophenol does not elicit an increase in respiratory rate when applied directly after treatment with victorin or dinitrophenol, but it causes a respiratory increase if applied 12 or 24 hours after the first treatment.

The ascorbate oxidation of homogenates prepared from victorin treated tissues was reduced considerably by addition of trichloracetic acid, not at all by the metal complexors, potassium cyanide and diethyldithiocarbamate, and very little by sodium azide. No inhibitor of ascorbate oxidation was found in healthy homogenates. The ascorbate oxidation of susceptible controls, boiled homogenates and homogenates prepared from resistant plants appeared to be the same. Steam pretreatment greatly increased the rates of ascorbate oxidation of both resistant and susceptible plants.

Autoxidation of ascorbate was stimulated somewhat by the addition of victorin.

Glycolic acid oxidation and α-ketoglutaric dehydrogenase activity were not demonstrated by the techniques used.

INTRODUCTION

Until comparatively recent times, investigators in the field of plant pathology have been concerned primarily with such morphological aspects of plant diseases as the description of visible symptoms, the identification of the pathogens and the testing of available materials for control of diseases. At present, this trend is being supplemented by investigations concerned with the biochemistry of host-parasite relationships. An accurate description of visible symptoms induced by pathogens is still of major importance to the plant pathologist, however, this examination has been widened to include the metabolic alterations that may occur in advance of, and lead to visible symptoms. One metabolic feature of diseased plants that has been and is being reported frequently, is an increased respiratory rate.

The reports of several investigators on the specific activity of victorin, a toxic metabolite found in culture filtrates of Helminthosporium victoriae, the causal organism of Victoria blight of oats, has given additional support to the toxin theory of pathogenicity. It has also provided a useful means for the study of metabolic changes induced in susceptible plants without the interference of the parasite. The respiratory changes induced in susceptible oats when treated with victorin suggest that similar responses may be found in naturally infected plants or in plants inoculated with H. victoriae. In the present study attempts were made to determine whether respiratory responses similar to those induced by victorin

could be found in plants inoculated with H. victoriae. Further attempts were made to clarify the relationship of ascorbic acid oxidation to the disease syndrome, and to investigate the possibility of respiratory uncoupling as an explanation for the increase in respiration induced by victorin.

REVIEW OF LITERATURE

It is probable that not all of the metabolic alterations found in diseased plants are of great importance. However, a survey of such changes should distinguish those that are most significant and characteristic of diseases in general. According to Allen (4, 5), Farkas and Kiraly (14) and Uritani and Azakawa (60) an increased respiratory rate may be considered a general response of plant tissues that are attacked by pathogenic microorganisms and viruses.

Most of the reports of pathogen-induced respiratory increases have appeared during the last ten years. But according to Farkas and Kiraly (14) the observations and conclusions of Doby, almost 50 years ago, might be considered the first attempt to correlate disease development and accelerated respiration. Doby noted that potatoes infected with potato leaf roll virus were low in carbohydrates and proteins, and concluded that an increased respiratory rate was responsible for these deficiencies. Ten years later in 1921, Weimer and Harter (65) reported that the respiratory rate of sweet potatoes infected with Rhizopus soft rot was nine times that of healthy tissues. In the reports of Allen (3, 4) on the altered respiration of wheat leaves infected with powdery mildew, an increase in respiration was observed. This increase was observed in tissues not penetrated by the fungus. The elaboration of a toxin was suggested to account for this increase but none was found. A few years later a toxic material was found in barley infected with powdery mildew (35). In an analysis of his work and the work of Sempio (51), Allen (4, 5) suggested the

means by which a respiratory increase might occur.

Since Allen's investigation in 1942, a number of papers have appeared in which pathogen-induced respiratory increases have been reported. Uritani and Azakawa (60) have listed most of these in tabular form. Respiratory increases have been reported in barley (35, 36), cabbage (6, 48), cotton (24, 55), rice (59), safflower (11, 12, 13), sweet potato (2, 56, 61), wheat (13, 14, 20, 50), tomato (25, 26), tobacco (39, 40, 66, 67), bean (67), Irish potato (1, 57), oat (15, 23, 45), and water melon (37). Fungi as well as bacteria (25, 26) and viruses (39, 40, 62, 63, 66, 67) have been involved as causal agents.

A number of workers have attempted to determine the causes for respiratory increases and several mechanisms have been considered (3, 4, 10, 11, 12, 13, 14, 36, 52, 53, 60). One of the theories is the possible uncoupling of oxidative phosphorylation so that oxidation would no longer be limited by the concentration of ADP and inorganic phosphate (4, 36, 60). Another mechanism that has been suggested is the acceleration of ATP utilizing processes (60). The increased concentration of ADP resulting from ATP breakdown would permit the ADP requiring processes of respiration to proceed at a faster rate if no other factors were limiting. In either case the results would be the same, i.e., increased respiration. Several processes that would account for greater ATP utilization have been listed by Uritani and Azakawa (60). These are: 1) the accumulation, mobilization and synthesis of phosphorous compounds; 2) growth of host tissues; 3) synthesis of proteins and activation of enzymes. The suggestion that the acceleration of energy requiring processes that lead to ATP breakdown has received

the support of several workers. Shaw and Samborski (52) and Yarwood and Jacobson (69) have reported that phosphorous accumulates in diseased tissues. Shaw and Samborski ran a series of experiments using radioactive materials and found that an accumulation of glucose occurred at the sites of infection and this was related to increased respiration. This accumulation of glucose did not take place when respiratory inhibitors were used, or when the tissues were exposed to anaerobic conditions.

The relationship of cellular growth of the host to the respiratory increase has been investigated by Daly and Inman (10) and others (25, 26, 41, 49). Daly and Inman found that the hypocotyls of rust infected safflowers elongated twice as rapidly and showed a greater absolute weight and length than controls. This increased growth would require more energy, accelerate ATP breakdown, and increase the respiratory rate. Samborski and Shaw have used this same line of reasoning in their work and attempted to relate hypertrophy in rust infected wheat to the increased respiratory rate (50).

The activation of protein synthesis has been suggested and some evidence has been reported to support this theory. Robertson and Pearson (44) have reported a correlation between the synthesis of nitrogen compounds in ripening apples and the rate of respiration. In studies concerned with black rot of sweet potato (2, 61) and in sweet potatoes infected with Helicobasidium mompa (56), increased protein synthesis and increased respiration have been observed. An increase in water soluble protein has been reported in Irish potatoes infected with Phytophthora infestans (58).

The abolition of the Pasteur effect has been suggested by several

workers and many of their data seem to substantiate this idea (12, 13, 14, 53, 56). The Pasteur effect has been defined simply as the inhibition of glycolysis in living organisms by aerobic conditions (8). The amounts of CO_2 evolved under anaerobic and aerobic conditions are compared and if the ratio obtained gives a value of less than 0.3 the Pasteur effect is thought to be taking place. At present, the Pasteur effect is thought to be related to the availability of organic and inorganic phosphates. During rapid respiration oxidative phosphorylation occurs and much ATP is produced. This results in a depletion of DPNH, ADP and inorganic phosphates which are necessary in some of the glycolytic reactions, and an inhibition of glycolysis ensues. In addition, results with such materials as 2,4-DNP that stimulate oxygen uptake and at the same time inhibit the uptake of phosphorus, the formation of organic phosphates, and apparently the Pasteur effect, give substance to this theory.

Allen (4), Farkas and Kiraly (13), Daly, et al, (12) and others (53) have reported either a change in anaerobic/aerobic CO_2 evolution, or other data that might lead to a reduction of the Pasteur effect. Some workers have used this information to support the idea that an alternate pathway has been activated in diseased plants. Farkas and Kiraly have reported a lack of sensitivity of the augmented respiration of rust infected wheat to malonate (13). A similar observation was made by Krupka on oats treated with victorin (23). These observations may indicate that the Krebs' cycle, or some portion of the Krebs' cycle is being replaced in diseased plants, or that there has been a build up of succinate in diseased plants so that malonate is no longer an effective competitor for succinic dehydrogenase in the concentration used.

In addition to the abolishment of the Pasteur effect, Shaw and Samborski have reported a predominance of CO_2 evolution from carbon-1 when rust infected plants were supplied with radioactive glucose (53). These data suggest the activation of the hexose monophosphate shunt (pentose pathway, direct oxidative pathway). Daly et al., have also reported a decrease in the C_6/C_1 ratio with disease development on safflower. However, the shunt, if it is in effect may be taking place in the parasite rather than the host (12). Several reasons have been suggested for a shift in respiratory pathways. Respiratory pathways may change with the age of plants (17) and with auxin concentration (18). Auxin and other materials have been shown to have some effect on respiratory rates and they may also affect the resistant response of some plants (10, 54). Several metabolites of fungi and diseased tissues have been demonstrated to be inhibitory to respiration in one concentration and stimulatory in another concentration (49).

Activation of enzyme systems which may be present in healthy tissues but do not predominate, may account for increased respiration. Kiraly and Farkas (20), Nishimura (37), and Asada (7) have reported increased ascorbic acid oxidase activity in plants in which a respiratory increase was noted. Krupka (22) has reported a similar response in oats treated with victorin, a toxic metabolite produced by Helminthosporium victoriae. Kalyanasundaram (19), and Pushpanaden (43) have reported decreases in the ascorbic acid content of diseased plants. Uritani et al., have reported increased amounts of ascorbic acid in sweet potatoes infected with Ceratostomella fimbriata. However, they also report increased activity of polyphenol oxidase (61). Increased polyphenol oxidase activity has been reported in Irish

potatoes infected with Phytophthora infestans (67) and increased peroxidase activity has been reported in cabbage with Botrytis cinerea, or when a culture filtrate of the fungus was applied to cabbage (6, 48). The methods that are used to distinguish the enzymes that are thought to be involved in terminal oxidation involve the use of metal inhibitors, and inhibitors that are selective for iron (cytochrome system) and copper (polyphenol oxidase and ascorbate oxidase). The utilization of inhibitors should differentiate the metallic and non-metallic enzymes, and the different metallic groups. The utilization of different substrates should distinguish between the copper enzymes since ascorbate oxidase only oxidizes ascorbic acid. Although these methods are thought to be reliable, recently an ascorbate oxidase that is not sensitive to a metal inhibitor was reported (29, 64) and Woinio et al. have reported the existence of a copper containing cytochrome system (63).

The role of polyphenols may be important in a diseased metabolism since they have been reported as associated with diseased tissues (46, 47, 55, 57). The fungitoxic action of these materials has also been reported (2, 26, 46, 47). It is of interest to note that several of the intermediates of the hexose monophosphate shunt are thought to be precursors of polyphenolic compounds (60). When certain diseases are considered, the polyphenol-polyphenol oxidase system may be of greater importance to resistance and resistance and resistance reactions since susceptibility was induced in resistant potatoes by the addition of copper chelating compounds (60).

The results of much research have not yet indicated the predominant importance of any one mechanism in the induction of a

respiratory increase in diseased plant tissues and it is probable that many factors are involved.

In the study of Victoria blight of oats, the restriction of the causal organism to the lower parts of the plants in spite of the extensive foliar symptoms led to the postulation that a toxin was produced which caused the symptoms (32, 33). This postulation was substantiated (27, 28, 33). It was found that the toxin was as specific as the causal organism on different oat varieties. Moreover, Luke and Wheeler (29) found that pathogenicity of isolates could be correlated with toxin production. Studies performed by Romanko (45) indicated that respiratory increases of great magnitude were induced only in susceptible varieties of oats upon exposure to toxin. Little to no response was observed in resistant varieties. Toxin treated tissues respired at a rate more than double that of control tissues, as compared with increases of 15 to 50 per cent which have been observed in relation to other plantsdiseases (60). The response was considerably more than that induced by 2,4-DNP. The work of Krupka indicated that the increased respiratory rate was a function of toxin concentration (23). Krupka also reported a distinct inhibition of the increased respiratory rate by NaF. Inhibition by phenylthiourea and diethyldithiocarbamate was greater in toxin treated tissues than in controls, indicating an activation of copper containing enzymes. Increased ascorbic acid oxidation and decreased ascorbic acid content were observed (22, 23) but no increases in the activity of polyphenoloxidase, cytochrome oxidase, catalase or peroxidase were observed. The preliminary experiments of Grimm and Wheeler (15), who observed respiratory increases and increased ascorbic acid

oxidation in susceptible oats that were inoculated with a spore suspension of H. victoriae, substantiate the results of Romanko and Krupka and further serve to support the toxin theory of pathogenicity at least in regard to Victoria blight of oats.

MATERIALS AND METHODS

The methods used in this study were essentially modifications of those used by Krupka (22, 23). Isolates of H. victoriae were obtained from H. E. Wheeler. Samples of crude toxin were obtained from L. R. Krupka and Peggy S. Johnston, or prepared from culture filtrates of H. victoriae, according to the methods of Luke and Wheeler (28). A standard forty-fold dilution of the crude victorin (about 300 units/ml) obtained from Krupka was used in many of the toxin experiments. However, when new toxin prepared from culture filtrates proved to be stronger an eighty-fold dilution was used to give comparable activity. Unless otherwise noted, these were the concentrations used. Through the remainder of this paper the following abbreviations will be used: ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; DDC, diethyldithiocarbamate; DNP, 2,4-dinitrophenol; DPN, diphosphopyridine nucleotide; TPN; triphosphopyridine nucleotide.

The plants used in this study were of the varieties Victorgrain 48-93, a variety susceptible to H. victoriae, and Camellia, a resistant variety. They were grown in 10-inch, 12-inch or 4-inch pots in the greenhouse and either inoculated or harvested for toxin treatment 4 to 5 weeks after planting. Plants were inoculated with a spore suspension by means of a hypodermic syringe, and placed in a moist chamber for 24 or 48 hours. Control plants were inoculated with sterile water. Plants treated with victorin were cut in the greenhouse, recut under water in the laboratory and placed in the standard dilution of victorin. Deactivated victorin solutions were used as control treatments. When

DNP was used as a pretreatment the concentration used was 10^{-5} M. However, when it was tipped into Warburg vessels a final concentration of 2×10^{-5} M was used. In preliminary studies in which DNP was vacuum infiltrated into the tissue, inconsistent results were obtained with several dilutions and vacuum infiltration was not considered a satisfactory means of applying DNP. Unless otherwise noted, a 4-hour treatment was used during which cuttings were placed in the solutions and a frosted 100 watt lamp was placed about 18 inches from the plants to facilitate uptake of the solutions.

Preparation of whole tissue for respiratory studies

After treatment the second and third leaves from the bases of the culms were removed. The midvein was excised and the half leaves were cut into sections approximately one-half inch long. Approximately 150 mg (fresh weight) was placed in the main compartment of the Warburg vessels, into which 2 ml of 0.05 M phosphate buffer (pH 5.8) with 0.02 M sucrose had been pipetted. A folded paper crimp weighing 0.03 g and 0.2 ml of 20 per cent KOH were added to the center well. In experiments in which additional treatments were applied 0.5 ml of victorin, or 2,4-DNP or buffer of the proper dilution was added to the side arm. Readings were recorded at 15, 30, or 60 minute intervals after a 15 minute equilibration period. All tests were carried out at 25°C with air as the gas phase. Duplicate or triplicate vessels were used for all samples and all experiments were run at least 3 times. In those experiments in which a 12 or 24-hour time lapse occurred between the treatment and the determination of oxygen uptake the stems were recut under water and placed in water during this period.

At the end of this time the plants were retreated as before or a constant respiratory rate was established and a second treatment was applied by tipping in the proper solutions from the side arms of the Warburg vessels. During the 12 or 24-hour time lapse toxin treated plants wilted considerably so that distal portions appeared completely dry and dead. For this reason the lower halves of the leaves were used only and the upper halves were discarded. Manometric changes were converted to microliters of oxygen per gram of tissue (dry weight) per hour (QO_2).

Preparation of homogenates

Homogenates were prepared from toxin treated plants or inoculated plants and control plants. The deveined leaves were cut in sections one-fourth inch long and homogenized in a Servall Omnimixer for 5 minutes. One gram of tissue to 10 ml of buffer, or some multiple of this ratio was used in the preparations of homogenates. A beaker of chipped ice was placed under the cup of the mixer. Buffer solutions were chilled before using. The homogenates were strained through 4 layers of cheesecloth, and 2 ml was then pipetted into the main compartment of the Warburg vessels. One-half ml of substrate was added to the side arm.

Ascorbic acid oxidation

A 0.05 M phosphate buffer solution containing 0.02 M sucrose, with a pH of 5.8 was used in the preparations of homogenates. A 0.25 M solution of ascorbic acid adjusted to a pH of 5.8 was used as a substrate. When inhibitors were used 0.5 ml was pipetted into the second side arm. The final concentrations of the inhibitors in the reaction

mixture were: KCN, 0.0075 M; NaN_3, 0.005 M; DDC, 0.001 M, and the protein percipitant TCA, 5 per cent. In those experiments in which DDC was used the pH of all solutions was adjusted to 7.1. After a 5 to 15 minute equilibration period one 15 minute reading was taken before tipping in the ascorbic acid. For the most part, the substrate was tipped in and two 15 minute readings were taken before tipping in the inhibitor. In some experiments, however, substrate and inhibitor were tipped in together and the rate of oxygen uptake was compared with samples into which only the substrate had been tipped. Since the oxidation of ascorbate in control homogenates was not reduced to any great extent by protein precipitation with trichloroacetic acid, oxygen uptake was calculated on a dry weight basis rather than a nitrogen basis.

Several experiments were performed to compare the rates of autoxidation with that induced by treatment with victorin. Homogenates were prepared from treated and control tissues and portions of each were boiled for 2 minutes. After equilibration, an endogenous rate was determined and ascorbic acid was tipped into the vessels. After two 15 minute readings were recorded $CuSO_4$ (5 ppm or 50 ppm) was tipped in. The rates of ascorbate oxidation of homogenates prepared from resistant toxin-treated plants was also determined. The ascorbate oxidation of homogenates prepared from tissues treated with steam was determined 24 hours after the treatment.

An attempt was made to determine the effects of toxin on autoxidation of ascorbate. One ml of 0.25 M ascorbate was pipetted into the main compartment of Warburg vessels. An endogenous rate was determined after which $CuSO_4$ (final concentration, 5ppm) was tipped in. After one

10 minute reading 0.5 ml of toxin (diluted 1:40 or 1:100) was tipped in.

Glycolic acid oxidation

A 0.1 M phosphate buffer of pH 7.3 was used in the preparation of homogenates. The crude homogenate was centrifuged at 1000 X gravity in a refrigerated Spinco centrifuge maintained at from 0 to 2°C. Two ml of the supernatant was pipetted into the main compartment of the Warburg vessels. A 0.5 ml aliquot of 0.2 M glycolic acid adjusted to pH 7.3 was added to one side arm and the inhibitor of glycolic acid oxidation, $NaHSO_3$ (final concentration; 10^{-3} M) or KCN, which is stimulatory (final concentration; 10^{-3} M) was added to the second side arm.

Dehydrogenation of α-ketoglutaric acid

Homogenates were prepared as previously described, or by modifications of the methods of Millerd *et al.* (34) and Conn and Young (9). A 0.05 M buffer solution with 0.02 M sucrose was used in the preparation of dilute homogenates. One-half ml of 0.2 M α-ketoglutarate prepared in buffer and adjusted to pH 7.1 was pipetted into one side arm of the Warburg vessels and used as the substrate. One-half ml of ADP (final concentration; 2×10^{-4} M) was added to the other side arm.

According to the method of Millerd, *et al.* (34), 30 g of tissue (entire) were ground in a cold mortar with 10 g of sand and 40 ml of 0.1 M phosphate buffer, pH 7.1 with 0.4 M sucrose. The brei was strained through cheesecloth, and the solution was centrifuged at 500 X gravity for 5 minutes in a refrigerated Spinco centrifuge maintained at 0-2°C. The pellet was discarded and the supernatant was centrifuged at 10,000 X gravity for 15 minutes. The supernatant was

discarded and the pellet was suspended in 20 ml of the sucrose buffer solution, and centrifuged at 10,000 X gravity for 15 minutes. The pellet was resuspended in 3.5 ml of sucrose buffer and 0.5 ml was placed in the side arm of the Warburg vessels. The following materials were prepared in 0.05 M phosphate buffer with 0.3 M sucrose, and the final concentrations in the reaction mixture have been listed: α-ketoglutarate, 0.02 M; $MgSO_4$, 0.001 M; ATP, 0.001 M; NaF, 0.01 M. The enzyme preparation and the ATP were placed in separate side arms and tipped into the main well separately. The final volume of the reaction mixture was 3 ml.

The method of Conn and Young (9) was modified somewhat. One hundred g of tissue (entire leaves) were ground in a chilled mortar with 15 g sand and 100 ml of 0.1 M phosphate buffer, pH 7.3, with 0.5 M sucrose. Two hundred ml of sucrose buffer was added, the mixture filtered through 2 layers of cheesecloth, and centrifuged at 500 X gravity for 5 minutes. The supernatant was then centrifuged at 10,000 X gravity for 15 minutes, the pellet suspended in 20 ml of 0.01 M phosphate buffer with 0.3 M sucrose and centrifuged at 10,000 X gravity. The final pellet was resuspended in 6 ml of the second sucrose buffer mixture and 0.5 ml was used in the Warburg vessels. Cofactors, inhibitors and substrates were used in the following final concentrations: α-ketoglutarate, 0.02 M; AMP or AMP and ATP, or ADP, 0.005 M; DPN, 0.0001 M; $MgSO_4$, 0.002 M; malonate, 0.01 M; cocarboxylase, 0.00004 M; coenzyme A, 0.00004 M; NaF, 0.01 M; cytochrome c, 0.0001 M. All materials were prepared in the second sucrose buffer solution. The enzyme preparation and the AMP (AMP and ATP or ADP) were pipetted into separate side arms. After a 5 minute equilibration period the

endogenous rate was recorded and the enzyme preparation was tipped into the main well of the Warburg vessel. After one or two 10 minute readings were taken the AMP (AMP and ATP or ADP) was tipped in. Controls were run in which either the enzyme preparation or α-ketoglutarate or organic phosphate was left out. These experiments were repeated with uncentrifuged homogenates, or without inhibitors, or with TPN (final concentration; 30 u g/ml), glutathione (50 u g/ml) and dehydroascorbic acid (50 u g/ml) in place of cytochrome c (31) or in conjunction with cytochrome-c.

EXPERIMENTAL RESULTS

Respiratory response of oat tissue inoculated with H. victoriae

In this series of experiments plants were inoculated in the greenhouse, harvested daily starting with the day after inoculation, and prepared for the Warburg vessels. This experiment was performed 3 times and the data have been listed in Tables 1 and 2. In the first experiment, it was noticed that the respiratory rate of inoculated plants was quite high on the first day after inoculation, but this rate fell considerably on the second day. For this reason the spore suspension used in the second experiment was washed by filtering through cotton and the spores washed by centrifuging and resuspending 5 times, before being used as inoculum. It was thought that the initial respiratory burst on the first day in experiment no. 1 might be due to the presence of toxin in the unwashed inoculum. The results of experiment no. 3, in which unwashed spores were also used tends to confirm this idea. A considerable amount of fluctuation was observed from day to day in the respiratory response induced by H. victoriae, however, daily respiratory rates of the control tissue varied from day to day as well. This fluctuation in inoculated tissue was less apparent in experiment no. 2 and for this reason the data from this experiment were used for Figures 1-3. A gradual increase in respiratory rate was observed in inoculated tissue and this information has been plotted on a dry weight basis in Figure 1. Although respiratory increases were noticed on the first day after inoculation,

Table 1. Respiratory rate of inoculated and healthy tissue and their responses to DNP.

Days after inoculation	ul O_2/G Dry Wt/Hr Inoculated F Wt	D Wt	Control F Wt	D Wt	Per cent Control F Wt	D Wt	ul O_2/G Dry Wt/Hr Inoculated plus DNP F Wt	D Wt	Per cent increase F Wt	D Wt	ul O_2/G Dry Wt/Hr Control plus DNP F Wt	D Wt	Per cent increase F Wt	D Wt
						Experiment No. 1								
1	216	1771	135	1167	160	152								
2	238	1759	184	1538	129	114								
3	423	2436	226	1487	187	170								
4	303	1916	246	1502	123	128								
5	293	1920	176	1096	166	175	304	2009	7	5	293	1888	66	72
6	283	1837	196	1453	144	126								
9	294	---	168	---	175	---								
10	182	---	221	---	82	---								
						Experiment No. 2								
1	247	2577	222	2241	111	114	374	3874	51	50	378	3819	70	70
2	187	2014	225	2289	83	88	293	3184	56	58	378	3843	68	68
3	394	3761	257	2568	152	146	475	4503	23	19	371	4089	44	59
4	516	4420	239	2512	216	178	555	4503	7	2	313	4303	31	71
5	592	3584	261	2818	226	127	657	3989	10	11	480	5186	64	84
6	497	3564	270	2368	171	150	549	3945	10	10	373	3045	29	29
7	238	1669	251	1467	95	112	251	1760	5	5	309	1806	23	23
8	303	723	295	2087	103	32	382	913	26	26	453	3195	53	53
						Experiment No. 3								
1	225	1987	192	1702	117	117	330	3138	47	58	309	2733	61	60
2	411	2939	175	1253	234	234	562	4019	37	37	329	2350	88	87
3	347	2172	228	1711	152	127	376	2354	8	8	324	2435	42	42
4	303	1976	175	1255	172	157	391	2550	29	29	355	2224	85	77
5	333	2414	160	1008	208	239	---	---	--	--	---	---	--	--
6	471	3019	214	1964	220	153	---	---	--	--	---	---	--	--
7	389	2519	185	1152	210	218	---	---	--	--	---	---	--	--

Table 2. Ascorbate oxidation of homogenates prepared from inoculated control tissues.

Days after inoculation	Treatment		Per cent control
	Inoculated	Control	
	Experiment No. 1 ul O_2/G F Wt/Hr		
2	133	82	162
3	89	59	150
9	595	129	461
	Experiment No. 2 ul O_2/G D Wt/half hr		
1	342	321	138
2	760	644	118
3	418	306	137
4	271	121	224
5	1137	312	364
6	569	268	212
	Experiment No. 3 ul O_2/G D Wt/half hr		
1	272	162	168
2	275	321	86
3	653	162	403
4	648	152	426
5	1279	243	526
6	907	409	222
7	818	396	206

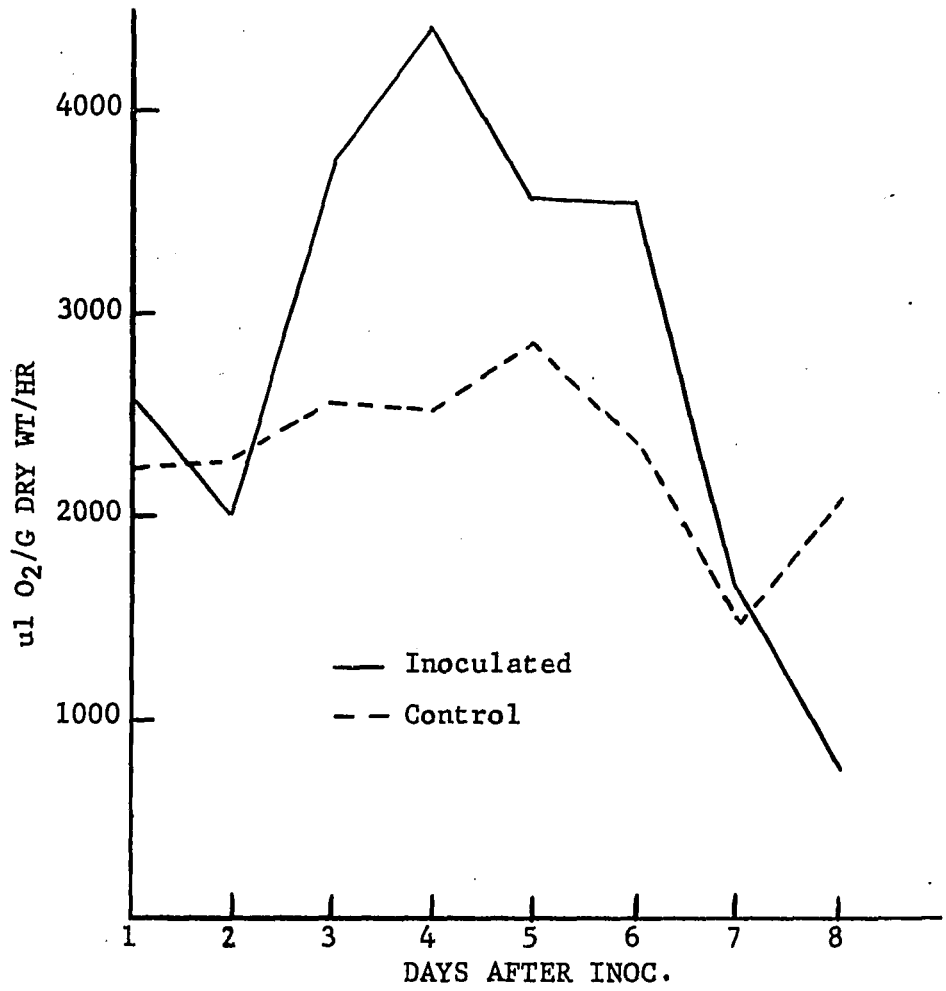

Figure 1. Respiratory rate of inoculated and control tissues.

macroscopic symptoms were not apparent until the third day, at which time incipient streaking was observed. Prominent striping of the leaves was observed on the fifth day. The respiratory peak was reached on the fourth day after inoculation, at which time the inoculated plants were respiring at a rate 80 per cent higher than the controls.

The data for the response of inoculated tissue to DNP from experiment no. 2 has been plotted in Figure 2. Here the respiratory increase induced by tipping in DNP varies with the response that has already been initiated by inoculation. Shortly after inoculation the response to DNP is still great, but not as great as that of control tissue. When the respiratory peak has been reached 4 days after inoculation, the response to DNP is almost nil. As the respiratory rate of inoculated tissue declines the response to DNP increases, but not to the extent of the control tissue.

The data obtained by Krupka (22) on the ascorbate oxidation of victorin treated tissues suggest that a similar response may take place in homogenates prepared from plants inoculated with H. victoriae. Ascorbate oxidation was measured daily in homogenates prepared from inoculated and control plants, starting from the day after inoculation. A low rate of oxygen uptake was observed before tipping in ascorbic acid. The per cent increase or decrease in relation to the endogenous rate was plotted and appears in Figure 3. A burst of activity was observed on the fifth day after inoculation, although ascorbate oxidation of homogenates prepared from inoculated tissue was always greater than that of the controls. It is of interest to note that the most rapid rate of ascorbate oxidation occurred one day after the respiratory maximum of whole tissue (dry weight), and Krupka reported a high rate

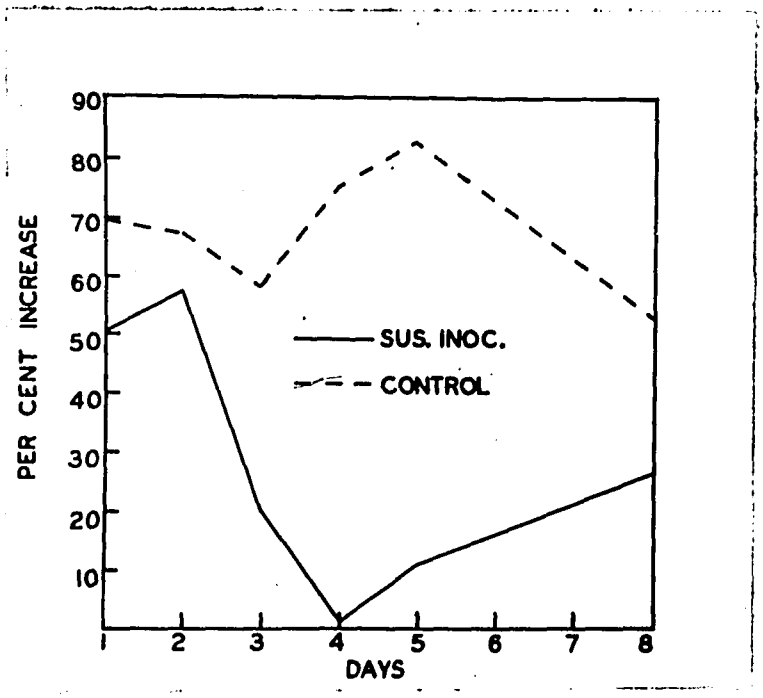

Figure 2. Respiratory response of inoculated tissue to 2,4-dinitrophenol.

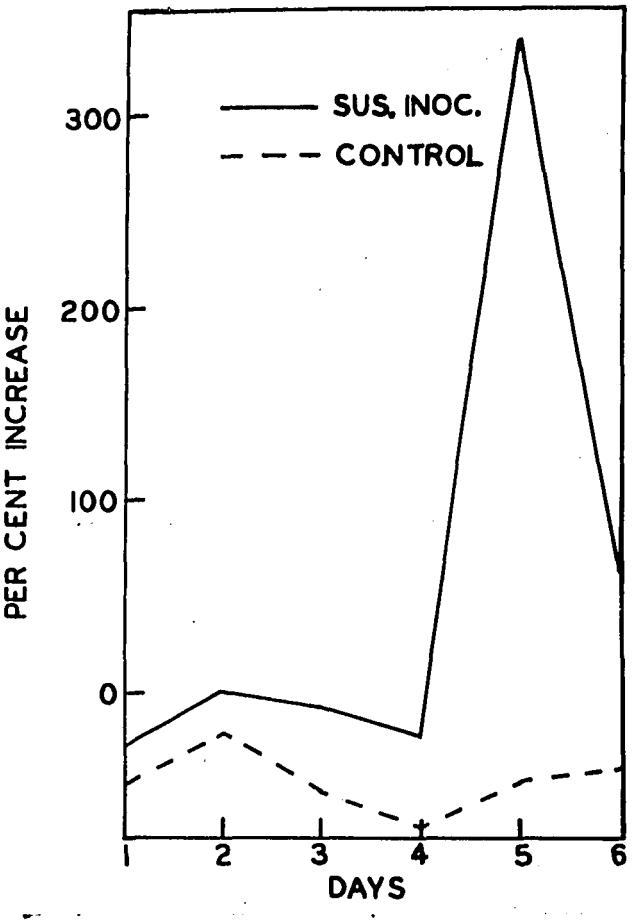

Figure 3. Ascorbic acid oxidation of homogenates prepared from inoculated tissues.

rate of ascorbate oxidation in homogenates prepared from victorin treated tissues, 12 hours after the treatment.

In spite of the fluctuations observed in respiratory response, response to DNP and ascorbate oxidation, it is apparent that a prominent respiratory increase takes place when susceptible oats are inoculated with H. victoriae, and a much smaller additional increase takes place when such tissues are treated further with DNP, as compared with control tissues. In addition, increased ascorbate oxidation takes place in homogenates prepared from inoculated tissues. Pathogenicity and toxin strength have been correlated by Luke and Wheeler (28), and toxin concentration and respiratory response have been correlated by Romanko (45) and Krupka (23). It is probable then that the slopes of the curves and the intensities of the peaks would vary with the pathogenicity of the isolate used for inoculation.

Clearly, these data extend the results of experiments with victorin treated tissues carried out by Romanko (45) and Krupka (23) to the diseased plant itself.

A comparison of the effects of victorin and DNP

The possibility that victorin has an uncoupling effect, and that this is responsible for the respiratory increase in victorin treated tissues has been suggested by Romanko (45) and Krupka (23) in Victoria blight of oats, and by others (4, 36, 60) in relation to other diseases. If uncoupling has already been induced by victorin, the addition of more victorin or another uncoupler might not be expected to produce further increases. Krupka reported that the addition of either victorin or DNP did not cause an appreciable increase in respiratory rate in tissues that had been previously treated with victorin.

In Table 3 the effects of tipping in additional DNP, on tissues previously treated with DNP, have been listed. Additional DNP applied while tissues were still under the influence of a pretreatment (4 hours) of DNP appeared to be inhibited somewhat, while the respiratory rate of tissues not pretreated with DNP increased by 46 per cent. Twelve and 24 hours after a pretreatment of DNP additional DNP induced an increase in respiratory rate. After 24 hours, the control plants that had been placed in water for this period appeared to be inhibited by the addition of DNP.

In Table 4 the results of tipping in victorin on tissue that had been inoculated and had reached the respiratory peak has been listed. At this time the addition of victorin appears to be only slightly stimulatory.

A number of experiments were performed to determine what effects victorin would have on tissues treated with victorin or DNP 12 or 24 hours before the second treatment. In addition DNP was tipped into vessels containing: (1) tissues in which uptake of victorin was induced twice with an interim of 12 or 24 hours; (2) tissues in which uptake of DNP was followed after 12 or 24 hours by uptake of victorin; (3) tissues which were placed in water for 12 or 24 hours and then allowed to take up victorin. The results of these experiments have been arranged in Table 5. Sixteen hours after an initial victorin treatment the respiratory rate dropped to less than one-third that of the controls. When such plants were again treated with victorin 12 hours after the initial treatment an increase in oxygen uptake of 54 per cent was recorded. When the second victorin treatment was applied by tipping the solution into the Warburg vessels an increase of more

Table 3. Effect of dinitrophenol (2 X 10^{-5} M) on the respiratory rate of tissues previously exposed to dinitrophenol.

First treatment	Interval*	QO_2		Per cent change
		No added DNP	DNP added	
DNP	0	3348	3195	- 5
DNP	12	2831	3031	+ 7
DNP	24	2544	3086	+21
Control (water)	0	1957	2531	+29
Control (water)	12	1778	2557	+44
Control (water)	24	2514	2072	-18

*Hours after first treatment before QO_2 was determined.

Table 4. Effect of victorin on the respiration of oats infected with H. victoriae.

Type of tissue		QO_2		Per cent change
		Without victorin	Victorin added	
Inoculated		2414	2696	+ 12
		3019	3304	+ 9
		2519	2140	- 16
	Average	2650	2713	+ 2
Control		1008	2004	+ 99
		1964	4276	+118
		1150	1509	+ 31
	Average	1374	2596	+ 89

Table 5a. Effect of various combinations of victorin and 2,4-dinitrophenol on respiratory rates of oat tissue. (The first and second treatments each consisted of 4 hours of uptake and were separated by a 12-hour interval. Materials used in the third treatment were tipped into the Warburg vessels.)

Treatments		QO_2 after second treatment	Third treatment	QO_2 after third treatment
First	Second			
Victorin	Water	517	Victorin	1051
Victorin	Victorin	797	DNP	1536
DNP	Water	1716	Victorin	2898
DNP	Victorin	2369	DNP	2277
Water	Water	1894	Victorin	2786
Water	Victorin	2052	DNP	2155
Water	Water	2091	Buffer	2369

Table 5b. Effect of various combinations of victorin and 2,4-dinitrophenol on respiratory rates of oat tissue. (The first and second treatments each consisted of 4 hours of uptake and were separated by a 24-hour interval. Materials used in the third treatment were tipped into the Warburg vessels.)

Treatments		QO_2 after second treatment	Third treatment	QO_2 after third treatment
First	Second			
Victorin	Water	351	Victorin	871
Victorin	Victorin	494	DNP	968
DNP	Water	1611	Victorin	2755
DNP	Victorin	2792	DNP	3246
Water	Water	1752	Victorin	2860
Water	Victorin	2391	DNP	2854
Water	Water	1851	Buffer	2338

than 100 per cent was noted. When DNP was tipped in on tissues twice treated with victorin an increase in oxygen uptake of 92 per cent was noted, however, here the increase may still represent a response to the victorin treatment. A 47 per cent increase was recorded in control plants when victorin was tipped in, but in control plants that were cut and allowed to take up victorin for 4 hours previous to determination of oxygen uptake, only a small increase was noted. Plants treated with DNP and then with victorin 12 hours later responded to the toxin, but additional DNP applied to these plants appeared to be inhibitory.

When these experiments were performed with tissues 24 hours after the initial treatment, similar responses were observed in most cases. Tissues which were treated with DNP or as controls and then treated with victorin 24 hours later showed a greater response when DNP was tipped in than those in the 12 hour series.

Ascorbate oxidation of inoculated and victorin treated tissues

Although the maximum in ascorbate oxidation did not occur until the fifth day after inoculation differences did occur between the rates observed in diseased and healthy homogenates. Krupka reported a rapid oxidation of ascorbate 12 hours after victorin treatment but information concerning ascorbate oxidation during this 12 hour period is lacking. For this reason a study was made of the rate of ascorbate oxidation at various times during the 12 hour period. Homogenates were prepared as previously described and ascorbate was tipped in and two 15 minute readings were taken.(Table 6). Trichloracetic acid, a protein precipitant, was then tipped in and the rate of reduction or stimulation was noted. A similar test was conducted with inoculated

tissues and the information has been listed in Table 7. On the first and second days after inoculation, and just after the victorin treatment the rate of ascorbate oxidation was somewhat higher than that of the controls, but the addition of TCA stimulated rather than inhibited ascorbate oxidation. At this time ascorbate oxidation appeared to be nonenzymatic. From the third day after inoculation and from 4 hours after victorin treatment TCA reduced the rate of ascorbate oxidation. On the fifth day after inoculation this inhibition amounted to 84 per cent, and at 9 and 12 hours after victorin treatment the rate was inhibited 57 per cent.

The rates of ascorbate oxidation in the control homogenates were always considerably less than in inoculated tissues, and the addition of TCA always stimulated the rates of oxidation. In the case of the deactivated toxin treated tissues, the addition of TCA reduced the rate by only 5 to 15 per cent at 9 and 12 hours after treatment.

Effects of respiratory inhibitors on ascorbate oxidation

Several respiratory inhibitors were used to determine what effects they would have on ascorbic acid oxidation of homogenates prepared from victorin treated tissue. In Table 8 the data have been listed. Potassium cyanide was stimulatory rather than inhibitory. Sodium azide inhibited ascorbate oxidation by only 10 per cent. Diethyldithiocarbamate appeared to have little effect. This test was run at a pH of 7.1 rather than at pH 5.8 because DDC is not stable at a slightly acid pH.

In order to determine whether an inhibitor of ascorbic acid oxidation might be present in homogenates of healthy tissues which victorin might have destroyed in treated tissues, a test was run in

Table 6. Ascorbate oxidation of homogenates prepared from victorin treated tissue.

Hours after treat.	Treatment: Victorin ul O_2/G D Wt/Half Hr AA	Victorin ul O_2/G D Wt/Half Hr AA plus TCA	Victorin Per cent change	Deactivated victorin ul O_2/G D Wt/Half Hr AA	Deactivated victorin ul O_2/G D Wt/Half Hr AA plus TCA	Deactivated victorin Per cent change
0	453	504	+11	227	357	+53
4	499	387	-33	334	520	+55
5	605	427	-30	287	373	+29
6	670	445	-34	304	482	+58
8	533	367	-32	205	295	+43
9	1480	642	-57	610	579	- 5
12	1220	527	-57	427	363	-15

Table 7. Ascorbate oxidation of homogenates prepared from plants inoculated with H. victoriae.

Da Days after inoc.	Treatment: Inoculated ul O_2/G D Wt/Half Hr AA	Inoculated ul O_2/G D Wt/Half Hr AA plus TCA	Inoculated Per cent change	Control ul O_2/G D Wt/Half Hr AA	Control ul O_2/G D Wt/Half Hr AA plus TCA	Control Per cent change
1	272	407	+49	162	308	+ 64
2	275	322	+17	321	477	+ 48
3	653	339	-48	162	307	+ 90
4	648	192	-70	152	288	+ 90
5	1279	205	-84	243	261	+ 7
6	907	753	-14	409	907	+121
7	818	349	-58	163	396	+142

Table 8. Effects of inhibitors on ascorbate oxidation of homogenates prepared from victorin treated tissues.

Inhibitor	ul O_2/G D Wt/Half Hr		Per cent change
	Without inhibitor	Inhibitor added	
KCN (7.5×10^{-3} M)	856	1095	+28
NaN_3 (5×10^{-3} M)	749	678	-10
DDC (10^{-3} M)	1149	1183	+ 3
Healthy homogenate	452	443	- 2

which 0.5 ml of healthy homogenate was tipped into vessels containing homogenates prepared from victorin treated tissues, after a rate of oxidation had been established. Little inhibition was observed (Table 8).

Similar rates of ascorbate oxidation were observed in homogenates prepared from control tissues and from boiled homogenates, as indicated in Figures 4 and 5. After tipping in $CuSO_4$ (5 ppm copper) all rates increased, but the rate of the boiled deactivated control increased tremendously. When 50 ppm of copper were tipped in similar results were obtained except that the rate of oxidation of the homogenate prepared from toxin treated tissues was somewhat reduced.

When the rates of ascorbate oxidation of homogenates prepared from resistant and susceptible tissues were compared some oxygen uptake occurred in all, but the addition of TCA did not inhibit the rates of resistant homogenates. The rate of the susceptible control was inhibited slightly (Figure 6).

The rates of ascorbate oxidation of susceptible and resistant homogenates 24 hours after steam treatment increased to about 4 times that of the endogenous rates. The rate in the susceptible control increased 166 per cent and the rate in resistant controls doubled (Table 9).

The autoxidation of ascorbate in the presence of 5 ppm copper appeared to be stimulated by the addition of toxin and slightly inhibited by the addition of deactivated toxin (Table 10).

Glycolic acid oxidation

Glycolic acid oxidation was run with homogenates prepared from toxin treated and control tissues directly after the treatment and 12

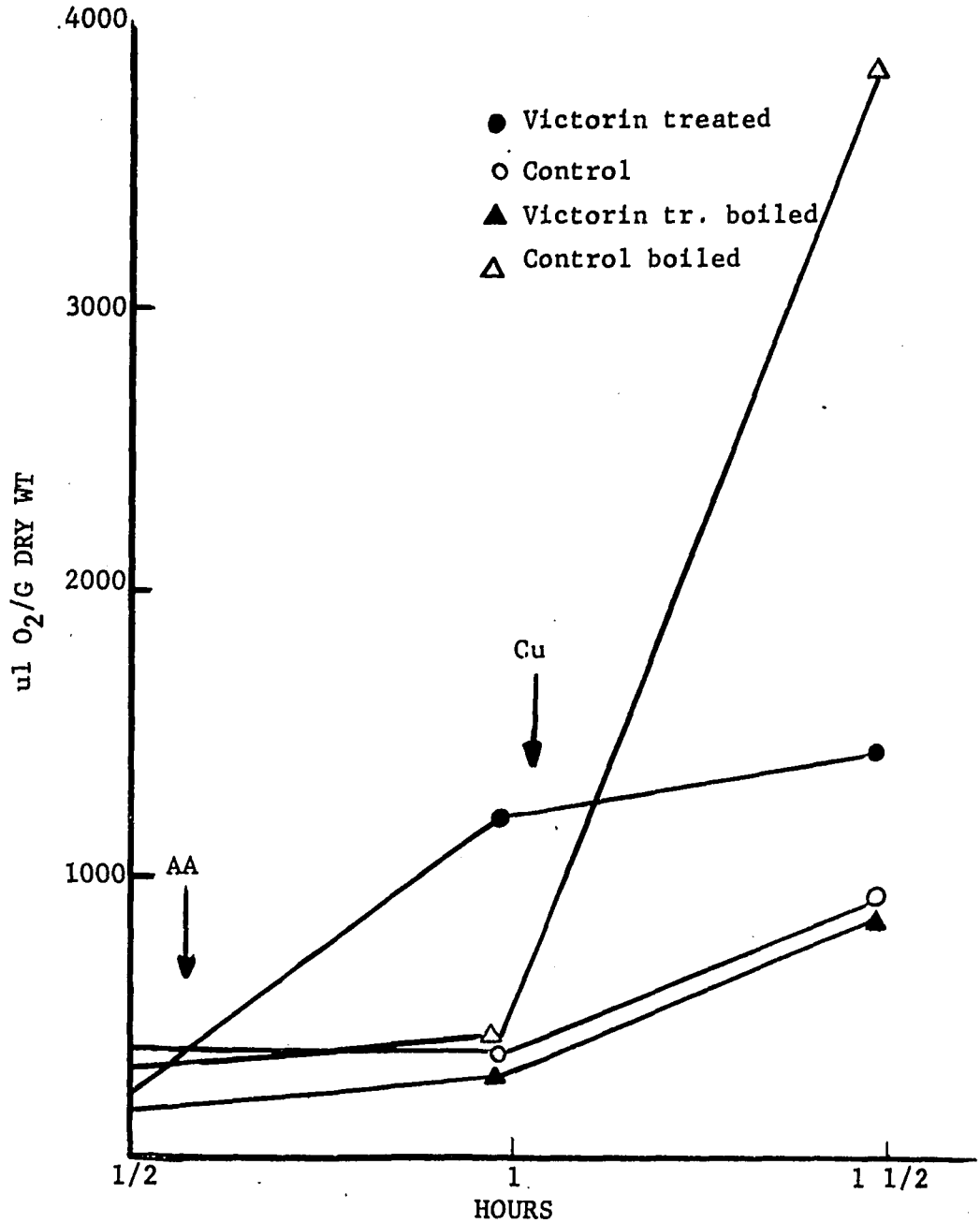

Figure 4. Ascorbate oxidation of boiled and unboiled homogenates with 5 ppm copper.

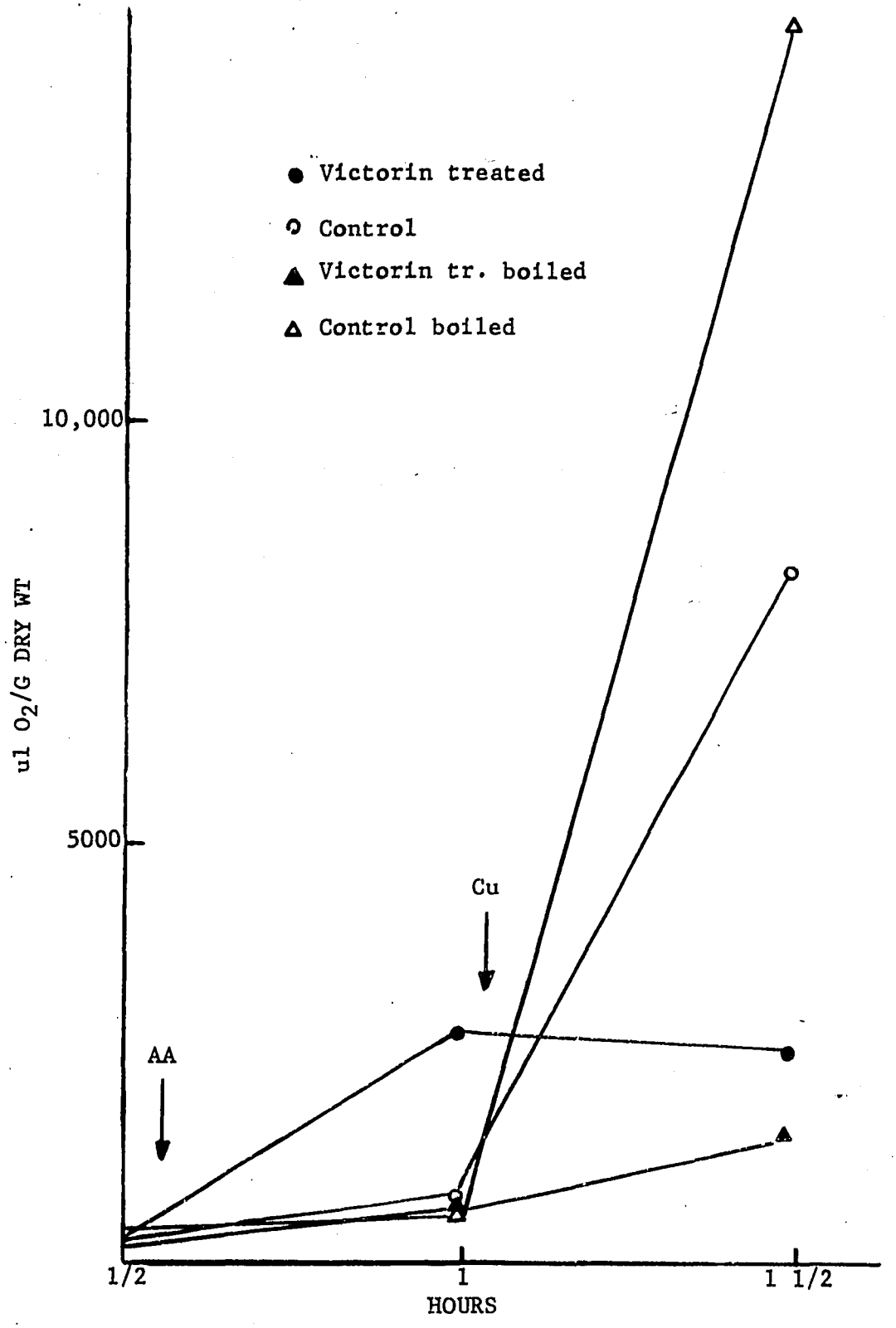

Figure 5. Ascorbate oxidation of boiled and unboiled homogenates with 50 ppm copper.

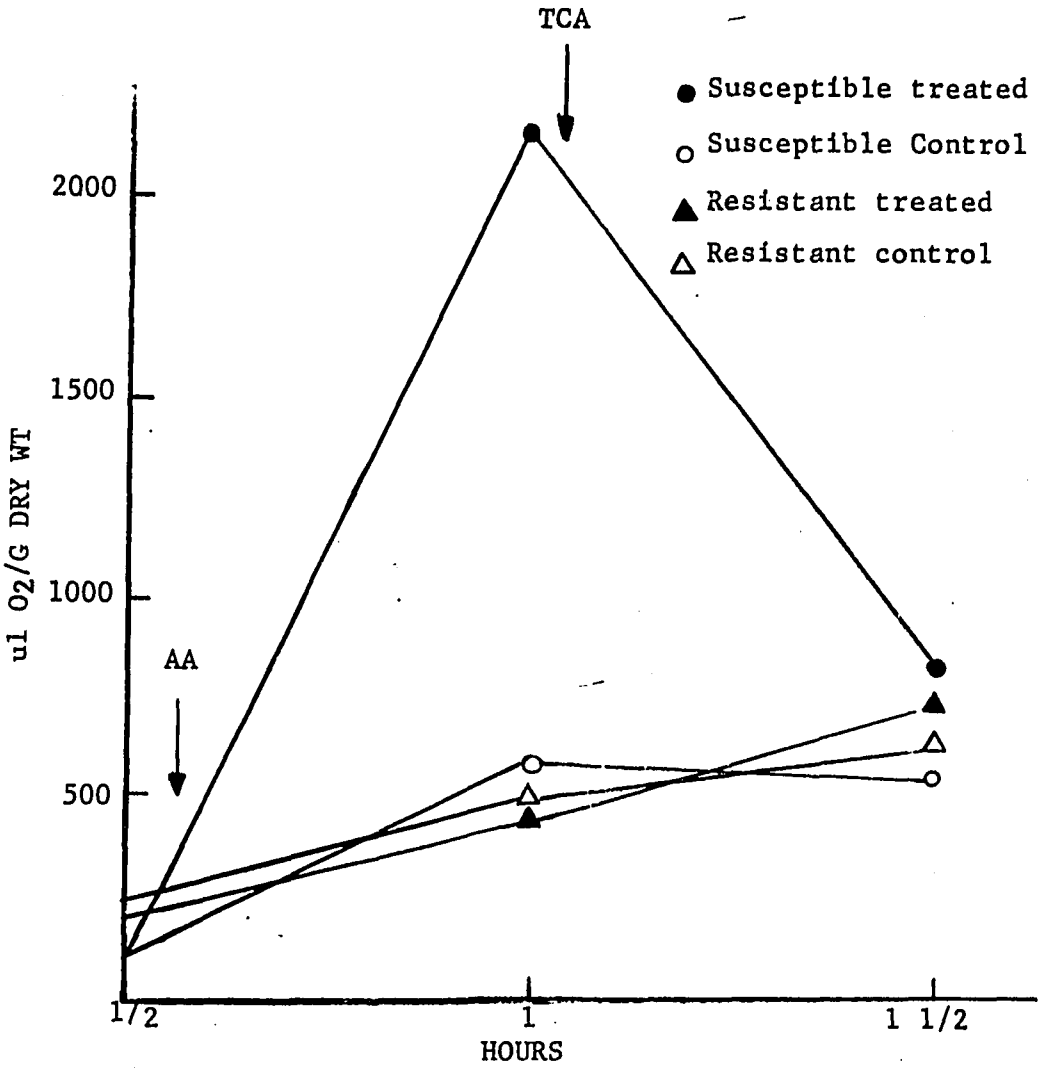

Figure 6. Ascorbate oxidation of homogenates prepared from susceptible and resistant oats.

Table 9. Ascorbate oxidation of homogenates prepared from susceptible and resistant oats 24 hours after treatment with steam.

Type of tissue	Q_{O_2} Endogenous	Q_{O_2} Plus ascorbate
Susceptible	219	869
Susceptible control	475	1268
Resistant	102	409
Resistant control	497	1009

Table 10. Effect of victorin on the autoxidation of ascorbate.

Treatments	ul O_2/10 minutes
Copper (5 ppm)	80
Copper (5 ppm), victorin (1:40)	110
Copper (5 ppm), deact. victorin (1:40)	72
Copper (5 ppm), victorin (1:100)	86
Copper (5 ppm), deact. victorin (1:100)	63

hours after the treatment. Glycolic acid oxidation was not observed under the conditions of these experiments.

Dehydrogenation of α-ketoglutarate

This series of exploratory experiments was run only with untreated tissues in order to determine whether dehydrogenation and decarboxylation of α-ketoglutarate were coupled to phosphorylation in oats as in other organisms. In other organisms the P/O ratio of this reaction is high. Future experiments were planned involving victorin and DNP treated tissues in order to determine whether this reaction could be uncoupled in oat homogenates by these materials. However, no great oxygen uptake was observed in samples containing the complete reaction mixture, and little differences were observed when these rates were compared to the controls, in which either the substrate or cofactor AMP (AMP and ATP or ADP) was omitted.

DISCUSSION AND CONCLUSIONS

The observations recorded here within confirm and extend the conclusions of Luke and Wheeler (29), Romanko (45), Krupka (23) and Grimm and Wheeler (15), and provide further evidence for Allen's toxin theory of pathogenicity, at least as far as Victoria blight of oats is concerned. The respiratory response in inoculated tissues, the reduction in response to DNP at this time, and the increase in ascorbic acid oxidation in homogenates prepared from inoculated tissues are all in agreement with Krupka's work with victorin. The data that were used to plot the curves in Figures 1, 2 and 3 showing these responses were obtained from experiment no. 2. Although the rates of increase, decrease in respiratory rate, response to DNP and ascorbate varied from one experiment to the next, the same general responses and patterns were observed in all experiments. This might be expected in view of the results of workers previously cited in which pathogenicity and toxin production and toxin concentration have been correlated. These observations also suggest that the use of a more standardized victorin solution might provide future researchers with more consistent data, since the variability inherent in living organisms would be restricted to the host tissue. At the same time this points up the necessity for a more complete characterization of the active components of

victorin, in order to be able to analyze its effects. Although Pringle and Braun (42) have done much to characterize this material, its structure and reactive groups are still not completely known.

Krupka reported that victorin or DNP produced little additional response in tissues previously treated with victorin. In the present study it was observed that inoculated tissues responded very little to toxin treatment at the time of maximum respiration. It was also observed that DNP stimulated tissues responded very little to the addition of more DNP, applied shortly after the first treatment. Although the mechanisms which induce these responses may not be the same, superficially these materials appear to produce the same results.

The respiratory rate of tissues that had been allowed to take up victorin, after which a period of 16 hours passed before the rate was determined dropped to less than one-third that of the controls. When a second treatment was applied by tipping in victorin the respiratory rate increased more than 100 per cent, but the actual rate was far below that of tissues treated just prior to the determination, and also below the rate of untreated controls. When the second victorin treatment was applied in the same manner as the first (4 hours uptake) they responded by an increase of 54 per cent. After tipping in DNP an additional increase of 92 per cent was observed. It is difficult to say whether this response was due to DNP or merely the continued effect of the second toxin treatment. It is of interest to note that tissues treated with water (controls)

and then allowed to take up victorin for 4 hours showed only a small increase over the untreated controls, and the addition of DNP to these tissues also produced very little response. When tissues were treated initially with DNP, 12 hours later the respiratory rate was similar to that of the controls. When these tissues were treated with victorin 12 hours after the initial DNP treatment an increase of 38 per cent in the respiratory rate was observed. After tipping in more DNP this rate was inhibited by 4 per cent. When the toxin treatment was applied by tipping in victorin an increase of 68 per cent was observed. This information is in contrast to that obtained from tissues which were allowed to take up toxin twice. It appears as if there has been a change in host response. Toxin treated tissues ordinarily do not respond to DNP directly after the toxin treatment, but after 2 treatments they respond to DNP. This suggests that there is more than one site of response in the host, or that uncoupling may have little to do with the respiratory response. If uncoupling took place initially an accumulation of phosphate acceptors might occur so that DNP, no matter when it is applied would cause no further response. Additional experiments should be attempted to clarify these data. The respiratory rate of control tissues increased by 14 per cent after tipping in additional buffer. No oxygen uptake was observed when twice treated tissues were boiled.

Similar results were observed when these experiments were run with a 24 hour interval between treatments. Control and DNP treated

tissues responded somewhat more to toxin treatment at this time. After tipping in DNP increases were observed that were slightly higher than those observed in the previous experiments. But control tissues showed an increase of 26 per cent after the addition of buffer, so the increases observed in controls and DNP treated tissues which were treated with toxin, then DNP, are probably of questionable significance.

Perhaps the main conclusion that can be drawn from these data is that there is never any real recovery to victorin treatment. The augmented respiratory rate of victorin treated tissues eventually drops below the level of control tissues, and although they will respond to more victorin or to DNP applied 12 or 24 hours after the initial treatment, the respiratory rate does not rise to the level of control tissues again. On the other hand, the respiratory rates of DNP treated tissues did not fall much below the respiratory rates of control tissues, 12 or 24 hours after the initial treatment, and a respiratory rate greater than that of the controls was induced by additional treatment. In this respect the effects of victorin and DNP appear to be quite different, however, the data presented here neither confirm nor deny Allen's suggestion that uncoupling of oxidative phosphorylation might account for the augmented respiration of diseased plants.

The inhibitory effect of water on oxygen uptake of tissues has been discussed by Ohmura and Howell (38). They found that water either in the Warburg vessels or soaking of tissues previous to oxygen uptake determinations had an inhibitory effect on oxygen uptake due to the

reduction in rate of oxygen diffusion. Just how important this may be in the experiments performed in this study is open to question.

In his review Mapson (30) has done much to consolidate and clarify the role of ascorbic acid and ascorbic acid oxidase in plant tissues. In addition, Mapson and Moustafa (31) have demonstrated the existence of a dehydrogenase-glutathione reductase-dehydroascorbic acid reductase-ascorbic acid oxidase terminal oxidase system in pea seedlings. Several reports of increased ascorbic acid oxidation in diseased tissues have appeared (7, 20, 37), and reports of decreased ascorbic acid content of diseased plants have also appeared (19, 43). Krupka (22) has reported both phenomena in victorin treated plants. The experiments performed for this study have yielded results that are somewhat conflicting. Increases in ascorbate oxidation were observed in inoculated tissues, and this information extends Krupka's results with victorin treated tissues to the diseased plant. In addition, the rate of ascorbate oxidation induced by inoculation was reduced by 84 per cent, and that induced by victorin was reduced by 57 per cent, by tipping in trichloracetic acid. It appears therefore, that the major part of ascorbic acid oxidation in diseased or victorin treated plants is enzymatically controlled. The results of the experiments with respiratory inhibitors seem to conflict with this idea. Cyanide and DDC had no inhibitory effect on the rate of ascorbate oxidation. Sodium azide inhibited the rate by only 10 per cent. Krupka obtained inhibition with azide and DDC, however, he vacuum infiltrated these materials into victorin treated tissues directly after the toxin treatment and inhibition of the respiratory increase of whole tissue was observed. In this study the inhibitors were applied to the homogenates

which were prepared 12 hours after the victorin treatment, at the time of rapid ascorbate oxidation. These results then, are probably not comparable to those obtained by Krupka. The inhibition with azide may be greater than it appears if 40 per cent of the rate of ascorbate oxidation is nonenzymatic. Both Mandels (29) and Ward (64) have reported the existence of an ascorbate oxidase that is resistant to cyanide, azide, diethyldithiocarbamic acid, sulfide, carbon monoxide in light and dark, 8-hydroxyquinoline, and other copper complexers.

Mapson (30) also refers to the works of those concerned with naturally occurring inhibitors of ascorbate oxidation in plant tissues. From the present study it does not appear as if such an inhibitor is present in control tissues, since the rates of ascorbate oxidation before and after the addition of control homogenates were approximately the same.

The rates of ascorbate oxidation of boiled control homogenates were the same as that of the unboiled control. From this it appears as if the oxidation of the control homogenate is, for the most part, nonenzymatic.

The rate of ascorbate oxidation of homogenates prepared from victorin treated resistant tissues did not exceed that of the control susceptible tissue, and these rates continued to rise after the addition of TCA. These data substantiate the conclusions concerned with the specificity of the toxin.

The rates of oxidation observed in homogenates prepared 24 hours after tissues had been exposed to a jet of steam were greater than the control rates regardless of the resistance of the variety. From this it appears as if increased ascorbate oxidation may be associated with

dying tissue, regardless of the cause.

Autoxidation of ascorbate by copper appears to be stimulated by victorin and inhibited slightly by deactivated victorin, however, the means for testing this response will have to be refined considerably before any general conclusions can be made.

The data concerning the effect of trichloracetic acid on ascorbate oxidation are somewhat confusing. In most cases the effect of TCA on check homogenates was to increase the rate, whereas it decreased the rate of oxidation in treated material. If a natural proteinaceous inhibitor were present in healthy homogenates the addition of TCA might be expected to destroy its effect and permit an increase in ascorbate oxidation, but the presence of an inhibitor or ascorbate oxidation in healthy homogenates has not been demonstrated. The reason for increased ascorbic oxidation of homogenates after tipping in TCA has not been determined.

Recently Zelitch (70, 71) has done much in the study of glycolic acid oxidation and its relation to respiration especially in connection with hydrogen transferring systems. Zelitch and Barber (71) have reported that glycolate oxidation of spinach particles is not a coupled reaction. If a change to such a system took place in diseased plants the effect would be comparable to uncoupling and an increase in respiratory rate might occur. With this in mind the experiments with glycolic acid were performed but the results were negative and glycolate oxidation was not observed. However, the experiments were not carried out with a concentrated particlate system, and more should be done before this possibility is abandoned.

Similarly, all experiments performed to determine the activity

of α-keto-glutaric dehydrogenase were negative. Here again, the lack of the proper technique may be the reason for the results obtained. Although the role of the Krebs cycle and Krebs cycle acids has been emphasized in a number of tissues (16), one cannot assume that the cycle is of equal importance in all organisms, although the universal nature of respiration can lead very easily to this generalization. If the glyoxylate cycle, proposed by Kornberg and Krebs (21) were activated in diseased tissues a large portion of the Krebs cycle would be replaced by an alternate route. Such a replacement might permit a more rapid rate of respiration if the steps in the Krebs cycle with high phosphate requirements have been replaced by uncoupled reactions. The small response of diseased or victorin treated tissues to DNP applied directly after victorin treatment or while diseased tissue is respiring at a rapid rate suggest this possibility. The low response of victorin treated tissues to malonate, observed by Krupka (24) also emphasizes the importance of investigating this possibility.

SUMMARY

The respiratory rate of tissues susceptible to H. victoriae increased after inoculation until a maximum rate was reached 4 days after inoculation. As the respiratory rate increased, a decrease in response to 2,4-dinitrophenol was noticed. Ascorbate oxidation reached its maximum 5 days after inoculation.

Plants that were treated with victorin, then retreated 12 or 24 hours later responded to DNP applied after the second victorin treatment.

Ascorbate oxidation in homogenates of victorin treated tissues and inoculated plants was reduced by 57 and 84 per cent, respectively, by TCA, but potassium cyanide and diethyldithiocarbamate had no inhibitory effects. Sodium azide was slightly inhibitory.

Homogenates prepared from healthy tissue had no inhibitory effect on ascorbate oxidation of homogenates prepared from victorin treated tissues.

The rates of ascorbate oxidation of boiled homogenates and homogenates prepared from treated resistant plants appeared to be the same as that observed in homogenates prepared from susceptible control plants.

Copper at 50 ppm inhibited ascorbate oxidation of homogenates prepared from victorin treated tissues.

The rates of ascorbate oxidation of homogenates prepared from both resistant and susceptible plants 24 hours after exposure to stem were considerably greater than the rates observed in untreated controls.

Autoxidation of ascorbate by copper appeared to be stimulated somewhat by victorin.

Tests for glycolic acid oxidation and α-ketoglutaric dehydrogenase activity gave negative results.

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VITA

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Title of Thesis: Respiratory Changes Associated with Victoria Blight of Oats

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